PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 15 May 2001 (15.05.01)	
International application No. PCT/US00/21848	**Applicant's or agent's file reference** 03230006TA
International filing date (day/month/year) 11 August 2000 (11.08.00)	**Priority date** (day/month/year) 13 August 1999 (13.08.99)
Applicant CARR, Marcus, E., Jr. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

08 March 2001 (08.03.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: MICHAEL E. WHITHAM
MCGUIRE WOODS
1750 TYSONS BLVD.
SUITE 1800
MCLEAN, VA 22102

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 03230006TA	Date of Mailing *(day/month/year)* 27 DEC 2000 **FOR FURTHER ACTION** **See paragraphs 1 and 4 below**
International application No. PCT/US00/21848	International filing date *(day/month/year)* 11 AUGUST 2000
Applicant HEMODYNE, INC.	

1. [X] The applicant is hereby notified that the international search report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Dorothea Laurence for Telephone No. (703) 308-1235

Form PCT/ISA/220 (July 1998)☆ *(See notes on accompanying sheet)*

PATENT COOPERATION TRE

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 03230006TA	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US00/21848	International filing date *(day/month/year)* 11 AUGUST 2000	(Earliest) Priority Date *(day/month/year)* 13 AUGUST 1999
Applicant HEMODYNE, INC.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (See Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21848

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/00, 38/48; C12Q 1/56, 1/68; G01N 33/86
US CL :424/94.64; 435/6, 13; 436/69; 514/2, 12, 18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.64; 435/6, 13; 436/69; 514/2, 12, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,205,159 A (CARR, JR.) 27 April 1993.	1-11
A	US 5,293,772 A (CARR, JR.) 15 March 1994.	1-11
A	US 5,691,160 A (JANMEY et al.) 25 November 1997.	1-11
A	CARR, M. E. Fibrin Structure and Concentration Alter Clot Elastic Modulus But Do Not Alter Platelet Mediated Force Development. Blood Coagulation and Fibrinolysis. 1995, Vol. 6, No. 1, pages 79-86.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 14 NOVEMBER 2000	Date of mailing of the international search report 27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21848

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	REID, T. J. A Method for the Quantitative Assessment of Platelet Induced Clot Retraction and Clot Strength in Fresh and Stored Platelets. Vox Sanguinis. 1998, Vol. 75, No. 4, pages 270-277.	1-11

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."
2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."
3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."
4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under Article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

The statement should be brief, it should not exceed 500 words if in English or if translated into English.

It should not be confounded with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It should not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

In what language ?

The amendments must be made in the language in which the international application is published. The letter and any statement accompanying the amendments must be in the same language as the international application if that language is English or French; otherwise, it must be in English or French, at the choice of the applicant.

Consequence if a demand for international preliminary examination has already been filed ?

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase ?

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

[P]ATENT COOPERATION TRE

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MICHAEL E. WHITHAM
MCGUIRE WOODS
1750 TYSONS BLVD.,LLP
SUITE 1800
MCLEAN, VA 22102-4215

WHITHAM, CURTIS & WHITHAM
RECEIVED
MAY 14 2001

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of Mailing *(day/month/year)* 09 MAY 2001
Applicant's or agent's file reference 03230006TA	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US00/21848	11 AUGUST 2000	13 AUGUST 1999

Applicant
HEMODYNE, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

Form PCT/IPEA/416 (July 1992) ★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 03230006TA	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/21848	International filing date *(day/month/year)* 11 AUGUST 2000	Priority date *(day/month/year)* 13 AUGUST 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant HEMODYNE, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 MARCH 2001	17 APRIL 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/21848

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-22 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 23-25 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-9 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/21848

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-11	YES
	Claims	none	NO
Inventive Step (IS)	Claims	1-11	YES
	Claims	none	NO
Industrial Applicability (IA)	Claims	1-11	YES
	Claims	none	NO

2. citations and explanations (Rule 70.7)

Claims 1-11 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the presently claimed method of identifying patients at risk for atherosclerosis by measuring platelet contractile force and clot elastic modulus.

---------- NEW CITATIONS ----------

NONE

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A61K 38/00, 38/48; C12Q 1/56, 1/68; G01N 33/86 and US Cl.: 424/94.64; 435/6, 13; 436/69; 514/2, 12, 18

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2001 (22.02.2001)

PCT

(10) International Publication Number
WO 01/12211 A1

(51) International Patent Classification[7]: A61K 38/00, 38/48, C12Q 1/56, 1/68, G01N 33/86

(21) International Application Number: PCT/US00/21848

(22) International Filing Date: 11 August 2000 (11.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/148,595 13 August 1999 (13.08.1999) US

(71) Applicant *(for all designated States except US)*: **HEMODYNE, INC.** [US/US]; 800 East Leigh Street, Suite 214, Richmond, VA 23219 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CARR, Marcus, E., Jr.** [US/US]; 2540 Swanhurst Drive, Midlothian, VA 23113 (US). **KRISCHNASWAMI, Ashok** [US/US]; San Jose, CA (US). **MARTIN, Erika** [US/US]; Richmond, VA (US).

(74) Agent: **WHITHAM, Michael, E.**; McGuireWoods, 1750 Tysons Blvd, Suite 1800, McLean, VA 22102 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/12211 A1

(54) Title: METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

(57) Abstract: Platelet contractile force and/or clot elastic modulus measurements are used to identify patients at risk for atherosclerosis or for bleeding during surgical procedures or other applications. Measurements which are elevated are indicative of atherosclerosis, and measurements which are reduced are indicative of a bleeding risk.

METHOD OF USING PLATELET CONTRACTILE FORCE AND WHOLE BLOOD CLOT ELASTIC MODULUS AS CLINICAL MARKERS

DESCRIPTION

BACKGROUND OF THE INVENTION

Field of the Invention

The invention is related to a method which uses platelet contractile force (PCF) measurements and/or clot elastic modulus (CEM) as clinical markers to allow rapid assessment of a patient's risk of atherosclerosis or a patient's bleeding risk during surgical procedures.

Description of the Prior Art

The interplay between atherosclerosis and thrombosis is complex. Multiple local and systemic thrombotic risk factors have been shown to play a role in the destabilization of the vulnerable plaque and its clinical sequelae. Aside from local factors such as the degree of plaque erosion or stenosis, well known systemic risk factors include cholesterol, diabetes mellitus, tobacco, cocaine, hypertension, elevated fibrinogen, impaired fibrinolysis, activated platelets and products or by-products of the coagulation cascade.

Platelet activation occurs in the acute coronary syndrome[1]. The acute coronary syndrome is a continuum from unstable angina to non-Q and

Q-wave myocardial infarction depending on the extent and duration of ischemia. Reduction in coronary blood flow occurs due to platelet aggregation, vasoconstriction at the site of coronary artery stenosis and endothelial injury. Endothelial injury may result from plaque ulceration, hemodynamic factors, systemic arterial hypertension, cardiac catherization, balloon angioplasty, etc. [2,3,4,5]. It is critical to recognize the acute coronary syndrome in patients who present to an emergency department with chest pain in order to prevent inappropriate discharge and adverse consequences[6,7].

Sensitive assays of individual components of the coagulation cascade have made laboratory evaluation of a biochemical hypercoagulable state possible. Prospective studies have suggested that elevated levels of factor VII, fibrinogen and other markers are associated with the development of ischemic cardiac events. However, traditional risk factors have not explained the increased cardiovascular risk in certain high risk groups such as diabetics. The contribution of platelet activation in patients presenting with an acute coronary syndrome has been well established. Unfortunately, to this point, tests of platelet function have not reflected changes predictive of a hypercoagulable state.

Platelet aggregometry, nuclear imaging techniques, serum markers such as Troponin I and T, P-selectin and E-selectin, intercellular adhesion molecules (ICAM) are some of the tools currently available and under investigation to identify patients with acute cardiac events. Nuclear imaging with technetium-99m sestamibi requires considerable resource utilization and has limited ability to differentiate between ischemia, ongoing infarction and prior infarction. Technetium-99m sestamibi also does not identify the unstable plaque [8,9,10]. Elevations of troponin in patients who have myocardial infarction excluded predict an increased risk for short and long term adverse cardiac events. Their utility in acute events is limited since

some degree of myocardial necrosis must occur prior to their release [11]. Platelet aggregation may be a useful marker for predicting mortality in coronary events [12]. However, aggregation techniques that have been used to evaluate platelet dysfunction have been limited to a few non-cardiac clinical situations [13]. Measurement of P-selectin [13], ICAM-1 and/or E-selectin [14] as early markers of platelet activation is ill suited to an emergency department setting because the techniques of flow cytometry and ELISA are time consuming, require technical expertise and need substantial dedicated equipment. Newer methods to assess platelet function are needed.

The Hemodyne® Hemostasis Analyzer is an instrument which measures platelet activity (platelet contractile force, PCF) and clot strength (clot elastic modulus, CEM) in physical units of dynes & dynes/cm^2 respectively[15,16]. U.S. Patents on which the Hemodyne® Hemostasis Analyzer is based include U.S. Patent 4,986,964, U.S. Patent 5,205,159, and U.S. Patent 5,293,772, and each of these patents are incorporated by reference in their entirety. Figure 1 schematically illustrates the components of a system similar to that described in these patents, and which is employed in the Hemodyne® Hemostasis Analyzer. A blood sample obtained from a patient is deposited in a sample cup 10 using a syringe 12 or other suitable device. The cup 10 is placed in a base 14, and a head piece 16 is inserted into the cup 10. This causes the blood 18 to distribute itself along the surface of the head piece 16 and up the sides of the cup 10. The force developed during contraction pulls the head piece 16 and base 14 closer together, and this force is measured using sensors connected to either or both the head piece 16 or base 14. To avoid adverse effects of the three dimensional structure on the clot during formation, a force can be periodically applied to the blood 18 during clotting by the head piece 16.

PCF and CEM are potentially useful tools in a variety of clinical situations[17,18,19]. PCF depends on thrombin production, platelet count,

platelet viability and the degree of platelet inhibition[15,20,21]. CEM depends on the fibrinogen concentration, fibrin structure and platelet function[15]. Inhibition of fibrin(ogen) binding to GP IIb/IIIa blockade either by disruption of GP IIb/IIIa or by competitive blockade, inhibits platelet mediated force development and results in clot structures which are substantially less resistant to deformation by outside forces[22].

Currently, a patient is screened for the presence of atherosclerosis by the patient's response to treadmill exercises and/or by cardiac catheterization. Both tests are time consuming and expensive, and catheterization is quite invasive to the patient. It would be helpful to have available a rapid, less invasive test which may identify those at risk for the presence of atherorsclerosis with the associated increased risk of adverse events such as myocardial infarction, peripheral vascular events, and stroke.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method which utilizes rapid recognition and quantification of platelet activation in patients to identify those at risk for adverse vascular events including thrombosis and hemorrhage.

There have been indicatons that PCF maybe elevated in patients with known coronary artery disease (CAD) when compared to normal control[23], and that CEM is elevated in CAD patients and is reduced, but not normalized, by aspirin therapy (Figures 2 and 3 show data illustrating these phenomena). Thus, it is widely acknowledged that platelets play a major role in arterial thrombosis and are thought to be pivotal in the pathogenesis of atherosclerosis. Despite these acknowledged relationships, no laboratory parameter has been demonstrated to be of value in the determination of thrombotic tendency due to platelet activity. Platelet count, the most

commonly measured platelet parameter, does not correlate with thrombotic risk. It is well known that high platelet counts do not imply an increased risk of thrombosis. Other common tests of platelet functions such as the bleeding time and platelet aggregation studies do not correlate with bleeding or thrombotic risk.

This invention provides a methodology where PCF and CEM are used to rapidly assess the risk of a patient for thrombotic events associated with atherosclerosis or with the risk of bleeding associated with deficient platelet function. Prior studies have not demonstrated that these measures could be used effectively as a screen for probable patient risk. In this invention, it is demonstrated that there is a statistically relevant correlation between PCF and/or CEM and thrombotic risk in patients with atherosclerosis. It is also demonstrated that there is a statistically relevant correlation between PCF and/or CEM and a patient's bleeding risk.

In the emergency department, the measurement of PCF and CEM could be used to detect evidence of hyper-platelet function associated with atherosclerosis in patients presenting with chest pain. Since the presence of atherosclerosis is the greatest risk factor for having a myocardial infarction, this piece of clinical evidence could be used to triage patients toward admission to the hospital or discharge to home. If the force is low or normal, the patient is less likely to have atherosclerosis and is therefore at lower risk of having an myocardial infarction. If the force is elevated two standard deviations above normal, the patient is at high risk even if the clinical history is not compelling.

While PCF and CEM will not diagnosis myocardiaol infarction, they do help identify the most important risk factor and therefore aid in the decision to admit and treat. This is a time consuming and expensive process in the emergency department. Despite the expense and effort, patients are sent home from emergency rooms every day in the United States who are

having a myocardial infarction. Some of these patients die of their event. Conversely, millions are spent admitting and monitoring patients who are not having a mycardial infarction.

Virtually all therapies used in the acute treatment of unstable angina and myocardial infarction result in a decline in PCF and CEM. Heparin anticoagulation, blockade of the platelet receptor glycoprotein IIb/IIIa (by Reopro, Integrilin or Aggrastat), and infusion of nitroglycerin all decrease PCF and CEM. Thus these parameters are not only useful in the identification of high risk patients, they can be used to monitor response to therapy

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of the preferred embodiments of the invention with reference to the drawings.

Figure 1 is a schematic diagram of measurement system used to monitor platelet contractile force and clot elastic modulus during clot formation in whole blood. Anticoagulated whole blood is placed in a shallow conical cup and clot formation is initiated by the addition of clotting agent. Prior to clot formation a conical upper plate is lowered onto the upper surface of the sample, trapping the sample between parallel surfaces separated by a known distance. Platelets within the sample attempt to collapse the clot resulting in a downward force on the upper platelet. This downward force is continuously monitored and the elastic modulus of the forming clot is intermittently measured.

Figure 2 is a graph which shows that preoperative platelet contractile force (PCF) is elevated in patients with documented coronary artery disease

(CAD) who are undergoing coronary artery bypass grafting (CABG). The forces are higher in all such patients but are much higher in such patients who are not taking aspirin. Aspirin appears to decrease but does not normalize PCF values.

Figure 3 is a bar graph which shows the effect of aspirin on whole blood clot elastic modulus (CEM) in patients with documented CAD who are undergoing CABG. CEM were measured at the time of maximal clot retraction. Values for patients with CAD taking or not taking aspirin were significantly elevated over those of asymptomatic control volunteers ($p<0.0002$).

Figure 4 is a bar graph which shows PCF is elevated in patients presenting in the emergency department with a complaint of chest pain. Samples for PCF and CEM determinations were obtained from 99 such patients soon after their arrival in the emergency room. The technician performing the assays did so without knowledge of the patient's clinical status. Upon presentation patient PCF values were significantly higher ($p=0.000000449$) than those seen in 50 asymptomatic volunteers.

Figure 5 is a bar graph which shows PCF values increase with the severity of the patient's clinical presentation. While all groups of patients had significantly elevated PCF values, those patients with electrocardiographic evidence of cardiac ischemia (levels II and I) had the highest PCF levels.

Figure 6 is a bar graph which shows CEM is elevated in patients presenting in the emergency department with a complaint of chest pain. Upon presentation patient CEM values were significantly higher ($p=0.0000145$) than those seen in 50 asymptomatic volunteers.

Figure 7 is a bar graph which shows PCF was significantly elevated in chest pain patients who are subsequently documented to have CAD ($p=0.0002$).

Figure 8 is a bar graph which shows CEM was significantly elevated in chest pain patients who are subsequently documented to have CAD (p=0.0041).

Figure 9 is a bar graph which shows PCF is significantly elevated in patients with hypercholesterolemia (p=0.00048).

Figure 10 is a bar graph which shows CEM is significantly elevated in patients with hypercholesterolemia (p=0.0398).

Figure 11 is a bar graph which shows PCF is significantly elevated in patients with diabetes mellitus (p=0.00012).

Figure 12 is a bar graph which shows CEM is significantly elevated in patients with diabetes mellitus (p=0.00037).

Figure 13 is a line graph that shows that in the chest pain study, PCF increased with age in both patient and asymptomatic males. The correlation was statistically significant (p=0.0032).

Figure 14 is a line graph that shows PCF increased with age in a separate study of apparently normal Italian males (p=0.0137).

Figure 15 is a line graph that shows that in the Italian study, PCF did not change with age in females under the age of 60.

Figure 16 is a line graph that shows that PCF increases with platelet count in all populations studied. The slope of the regression line allows calculation of an average force per platelet number for varying populations. Patients with known arteriovascular disease have higher force per platelet values than asymptomatic age matched controls (see table 3).

Figure 17 is a line graph that shows that collagen-induced whole blood platelet aggregation was depressed in patients presenting in the emergency department with a complaint of chest pain. Samples for aggregation were obtained from 99 such patients soon after their arrival in the emergency room. The technician performing the assays did so without knowledge of the patient's clinical status. Upon presentation patient

aggregation values were significantly lower (p=0.000000449) than those seen in 50 asymptomatic volunteers. While all groups of patients had significantly decreased aggregation, aggregation did not vary significantly between the varying risk levels.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The invention contemplates making PCF and/or CEM measurements on whole blood clots obtained from patient samples during clot formation, and then using these measurements as a screen to identify patient's at risk for an adverse vascular outcome. Application of this technique to clinical samples confirmed that clots with low PCF and/or CEM were less hemostatic and placed the patient at risk for bleeding in conditions such as primary fibrinolysis, Glanzmann thrombasthenia and coronary artery bypass procedures. PCF values less than 4 kilodynes after 720 seconds of clotting are abnormally low. Patients with severe thrombasthenia typically have PCF values below 2 kilodynes. CEM is affected by both fibrinogen concentration and platelet function. CEM values less than 14 kilodynes per cm^2 are indicative of deficient clot formation. In addition, application of this technique to clinical samples confirms that elevations of PCF and CEM are associated with arteriovascular disease and increased risk of arterial thrombosis. Specifically, patients with coronary artery disease, hypercholesterolemia, and diabetes mellitus have much higher PCF and CEM values than asymptomatic controls. In addition, patients who present to the emergency department with complaints of chest pain have significantly elevated forces and the degree of elevation increases with increasing clinical risk. PCF increases with age in males. However, while slightly higher in young females than in young males, PCF does not increase

with age in females at least to the point of menopause. Elevated whole blood PCF and CEM values should help identify patients at increased risk of arterial thrombosis due to atherosclerosis and enhanced platelet function. These measurements should prove useful during the triage of chest pain patients in the emergency department as well as the screening of asymptomatic patients with positive family histories or other documented risk factors for atherosclerosis. Since most therapeutic measures used to acutely treat arterial thrombosis reduce PCF and/or CEM, these parameters can also have applications as monitors of clinical response.

Screening of asymptomatic individuals with PCF and CEM measurements could be useful in indentifying patients who might benefit from more invasive and expensive testing. This can be accomplished by testing a small sample of venous blood. If the PCF value is greater than one standard deviation above the mean of normals, greater than 8.5 kilodynes and the patient has a positive family history or other risk factors (diabetes, cigarette smoking, hypercholesterolemia, etc.), then they should undergo additional testing. If the PCF is normal, 6.9 ± 0.7 kilodynes, no additional testing is needed. If the PCF is above 7.6, testing at intervals to assess whether the force is increasing would be appropriate.

Methods

Patient selection

All patients who present to the emergency department (ED) of the Medical College of Virginia/Virginia Commonwealth University (MCV) with symptoms suggestive of cardiac ischemia undergo prompt clinical evaluation which includes a history, physical exam and ECG. 99 patients presenting to ED with chest pain were recruited for this. When appropriate, blood samples, EKG and a brief history were performed by the ED nursing staff prior to the ED physician interview. Blood samples were obtained prior to the initiation of any therapeutic measures. Further management including

early perfusion imaging with technetium-99m was based on the discretion of individual ED physician and a well established chest pain protocol[24]. Table 1 sets for the acute cardiac evaluation and therapy guide under the protocol.

Table 1. Acute Cardiac Evaluation and Therapy Guide

Diagnosis	Treatment
Level 1	
Acute Myocardial Infarction	
ST elevation	t-PA or primary PTCA
Posterior MI	admit CCU
LBBB with strong clinical Suspicion for AMI	
Level 2	
Unstable Angina	
Ischemic ST-depression or	Standard USA protocol
Ischemic T-wave inversion	
Acute onset CHF	
Known CAD with typical symptoms	
Level 3	
Probable Unstable Angina	
Non-ischemic ECG &	Imaging with Technetium–99m
Typical CP>30 minutes	CCU fast-track
Atypical CP > 30 minutes with	If rest nuclear imaging positive admit as level 2
Multiple risk factors	If negative –stress cardiolite - ASAP
Level 4	
Possible Unstable Angina	

Nonischemic EKG & Brief typical chest pain or	Rest imaging with Technetium –99m If negative – home with f/u stress in am
Prolonged atypical CP or Cocaine CP	If positive – CCU admit –treat as level 2

Level 5

Noncardiac CP with clear-cut diagnosis	As appropriate

The hospital course for admitted patients was followed for pre-selected endpoints.

Forty-eight controls were also recruited and similar blood samples were obtained. Exclusion criteria for the control population included no current illness, no history of coronary artery disease or cerebrovascular accident, no recent ingestion of nonsteroidal inflammatory agents including aspirin. Samples for the individual tests were run soon after venipuncture. The institutional review board approved the study protocol.

Sample Handling

A single 15-ml blood sample obtained via an aseptic venipuncture prior to any therapeutic measure was placed into evacuated tubes containing 3.8% sodium citrate. Collagen-induced platelet aggregation, measurements of platelet contractile force (PCF) and elastic modulus (EM) were run in duplicates on whole blood.

Platelet aggregation

Platelet aggregation was measured utilizing a Chrono-Log® whole blood lumi-aggregometer. 450μL of citrated whole blood was mixed with 450 μL of saline and placed in an aggregometer cuvette equipped with a

stirring bar. Platelet aggregation was induced by the addition of collagen (3 mg/ml, Chronolog, Havertown, PA) and the change in impedance was monitored for six minutes.

Measurement of Platelet Contractile Force and Clot elastic Modulus/ Clot formation

Human thrombin, greater than 90% alpha, was purchased as a lyophilized powder from Sigma Chemical Co. (St. Louis, MO). The material with a specific gravity of 3000 NIH units/ml was dissolved in water, diluted with 0.10 M NaCl to a final concentration of 225 units/ml, divided into 50 µL lots and frozen at 80°C. Thrombin was free of plasmin and plasminogen. Nanopure water was used in the preparations of all solutions. Clotting was initiated by adding thrombin (1 NIH unit/ml) and calcium chloride (10mM) to 700 µL of whole blood. Force development was measured for 900 seconds.

The Hemodyne® RM-2 hemostasis analyzer (Hemodyne, Inc., Richmond, VA, USA) measures forces generated by platelets within a clot formed between two parallel cone-shaped plates (Figure 1). The temperature of the sample is held constant via thermal control of the bottom cone, which serves as the sample cup. Before gelation, the upper cone is centered above the cup and lowered into the clotting solution. As the clot forms, it attaches to the inner walls of the cup and upper cone. The entire sample volume is contained between the upper and lower surfaces. Once clotting is complete, platelets within the network pull fibrin strands inward transmitting force through the network to the surfaces to which the clot is adherent. Force measurement is accomplished utilizing a displacement transducer coupled to the upper cone. As platelets contract, the transducer produces an electrical output proportional to the amount of force generated.

Platelet contractile force is determined by measuring the amount of displacement (ΔV_2) of the upper cone during the course of the reaction. In order to compensate for the changing rigidity of the fibrin network, a calibrated compressive force ($F_{applied}$) is periodically applied to the sample by means of an electromagnetic solenoid, and the resulting voltage signal (ΔV_1), due to the displacement of the gel, is measured. PCF is then calculated as follows: PCF $= \Delta V_2 \times (F_{applied} / \Delta V_1)$

Clot Elastic Modulus (CEM) is obtained simultaneously with the PCF. The ratio of applied force (stress) to measured displacement (strain) is used to calculate the elastic modulus: CEM = stress/strain. Where stress equals the applied force ($F_{applied}$) divided by the area of application, and strain is the degree of shape change induced by the applied force. In the present case, the strain induced by $F_{applied}$ is measured as the change in gel thickness, which is the same as the change in the gap between the two cones. Strain is recorded as the ratio of the change in gap distance (d_1) to the original gap distance (d_0). Because the gel is a cylinder of radius (r) and length d_0, stress = $F_{applied} / pr^2$, strain $= d_1/d_0$ and CEM = $(F_{applied} / pr^2) / (d_1/d_0)$ The distance moved (d_1) is measured directly by the displacement transducer.

Sestamibi imaging and interpretation

Chest pain patients were injected with ~ 20mCi sestamibi in the emergency department (not more than 6 hours after the last episode of chest pain) as per the chest pain protocol. Perfusion images were evaluated by an experienced nuclear medicine attending physician and all data were made available to the physicians treating the physician. For purposes of this study, images were classified as either positive or negative for acute myocardial infarction (MI) or ischemia. A positive study required a discrete perfusion defect with associated abnormalities in wall motion and thickening. Studies

visually interpreted as normal, equivocal or consistent with cardiomyopathy were considered negative for acute coronary syndromes. Normal studies had normal perfusion and systolic function without regional wall motion or thickening abnormalities. Studies consistent with cardiomyopathy showed reduced systolic function on cinematic replay with either normal perfusion or perfusion defects without accompanying segmental wall motion abnormalities.

Endpoints

Patients who were admitted to the hospital were followed for specific endpoints. The primary endpoints were myocardial infarction, death, or urgent revascularization (coronary artery bypass graft surgery (CABG), or percutaneuous transluminal coronary angioplasty (PTCA) during the initial evaluation or within 5 days of admission.

Definitions

Myocardial infarction was defined as CK-MB mass $\geq$8.0 ng/dl with a relative index (CK-MB mass/total CK x 100) $\geq$ 4.0. For patients having both MI and revascularization, only MI was counted as an event. Anginal symptoms were considered typical if they were described as pressure, tightness, squeezing, burning, heaviness, crushing, or indigestion, or were similar to prior symptoms of angina.

Statistical analysis

Results were presented as mean value $\pm$ SD. Comparisons were made using the Student's t-test and chi-square analysis for categoric and continuous variables. A p-value < 0.05 was considered significant. The relative risk was calculated for various variable correlation coefficients.

Results

Baseline Demographics

The baseline demographics in the patients with chest pain and control patients are given in Table 2. The mean age was 52.8 ± 13.9 (23-87) in the chest patients as compared to 37.7 ± 10.1 (19-62) which was statistically significant. A significant difference in race and sex were also present. There were more blacks in the chest pain group and a preponderance of chest pain patients were male when compared to the control population. As expected, the chest pain patients had a greater number of traditional risk factors as compared to the control population.

TABLE 2

Baseline Demographics in Patients with Chest Pain and in Control Patients

	Chest Pain Patients	Control
Number	99	46
Age (years)	52.8±13.9 (23-87)	37.7±10.1 (19-62)
Gender		
Male	54(54.5%)	23(47.9%)
Female	45(45.5%)	25(52.5%)
Race		
Black	71(71.7%)	6(12.5%)
White	28(28.3%)	32(66.7%)
Asian		10(20.8%)
Smoking Status		
Current Smoker	39(39.4%)	3 (6.5%)
H/O Diabetes Mellitus	20(20.2%)	None
H/O Hypertension	14(14.1%)	2 (4.4%)
H/O Hypercholesterolemia	28 (28.3%)	1 (2.2%)
Mean Platelet Count ($x10^3$)	254±76(149-541)	257±53(181-367)

Mean Hemoglobin (g/dl)	13.2±1.8 (9.2-17)	
Mean time to sample run (mts)	149±83.9	91.5±68.3(10-345)

Risk Stratification/ Myocardial Infarction & Revascularization

All chest pain patients versus controls. PCF was significantly elevated (8.60±0.238 Kdynes) in patients presenting with chest pain as compared to controls (6.95±0.214 Kdynes)) (see Figure 4). PCF was highest in patients with more critical chest pain protocol levels (I&II), but was significantly elevated at all levels (see Figure 5). PCF for I & II level patients grouped together versus grouped III & IV levels approached but did not reach statistical significance (p=0.0735). CEM was also significantly elevated for all levels of chest pain patients compared to normals (Figure 6).

Patients with CAD versus controls. Thirty-six of the 99 patients were documented to have coronary artery disease (CAD) by cardiac catheterization or the occurrence of an acute clinical event. PCF was significantly (p=0.0002) elevated in these patients (8.87±0.459 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 7). CEM was significantly (p=0.0041) elevated in these patients (26.81±1.606 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 8).

Patients with Hypercholesterolemia versus controls. Twenty-eight of the 99 patients were documented to have serum cholesterols greater than 220 mg/dL. PCF was significantly (p=0.00048) elevated in these patients (8.68±0.434 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 9). CEM was significantly (p=0.0398) elevated in these patients (25.40±1.742 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 10).

Patients with Diabetes Mellitus versus controls. Twenty-five of the 99 patients were shown to have hemoglobin A1c levels greater than 7.0.

PCF was significantly (p=0.00012) elevated in these patients (9.57±0.591 Kdynes) compared to controls (6.95±0.214 Kdynes) (see Figure 11). CEM was significantly (p=0.00037) elevated in these patients (30.60±2.174 Kdynes/cm^2) compared to controls (22.08±0.588 Kdynes/cm^2) (see Figure 12).

Patients with Positive versus Negative Sestamibi. Sixty-four of the 99 patients underwent sestamibi scanning. Fifteen of these sixty-four patients had a positive scan. PCF tended to be higher in patients with positive (9.4±0.8 Kdynes) versus negative (8.2±0.3 Kdynes) although the difference did not reach statistical significance (p=0.08). Similarly, CEM tended to be higher in patients with positive (29.9±2.6 Kdynes/cm^2) versus negative (25.2±1.1 Kdynes/cm^2) although the difference did not reach statistical significance (p=0.07).

Patients with positive clinical endpoints. Seven patients (7.07%) were documented to have suffered a myocardial infarction (MI). An additional five patients (5.05%) underwent revascularization. Total group of MI and revascularization patients was twelve of ninety-nine (12.12%). PCF was significantly (p<0.05) elevated (8.2±0.7 Kdynes) in the positive endpoint group compared to normals (6.9±0.2 Kdynes). Similarly, CEM was significantly (p<0.05) elevated (24.9±1.9 Kdynes/cm^2) in the positive endpoint group compared to normals (21.7±0.6 Kdynes/cm^2).

Effect of Age. PCF increased with age when all males (patients and controls) were considered as one group (Figure 13). This result was confirmed in a smaller Italian study of asymptomatic males (Figure 14). PCF did not increase with age in American or Italian (Figure 15) females. It is to be noted that this result has only been confirmed in females below the age of fifty-five.

Platelet Force Per Platelet (FPP). PCF is dependent upon and increases with increasing platelet concentration (Figure 16). However, the

increased PCF values in chest pain patients were not due to elevated platelet counts (Table 2). Instead, the slope of the force versus platelet concentration plot (Figure 16) was increased in similar plots for CAD and DM patients. Such plots allows the calculation of a new parameter - force per platelet (FPP). Table 3 shows FPP was highly significantly elevated in CAD and DM patients relative to asymptomatic controls.

TABLE 3

Mean Platelet Contractile Force Per Platelet Values for Various Test Groups

Group	PCF/Platelet (Dynes x 10^{-5} / platelet)	p-value
Controls	3.97	
Coronary Artery Disease	5.309	0.000217
Diabetes Mellitus	6.19	0.000743

Platelet Aggregation in Chest Pain Patients versus controls. Collagen induced whole blood platelet aggregation was significantly reduced in patients presenting the emergency department with chest pain (Figure 17). However, the degree of suppression did not correlate with clinical risk levels.

Table 4 contains a complete odds ratio analysis for the chest pain study.

TABLE 4

Odds Ratio Analysis for PCF and EM versus known risk factors for atherosclerosis and coronary artery disease

	PCF		EM	
	OR(CI)	p value	OR(CI)	p value
CAD	2.0(0.5-7.4)	ns	1.3(0.4-4.6)	ns
DM	2.7(1.7-7.3)	0.06	2.7(1.7-7.3)	0.06
Hypercholesterol	1.6(0.6-4.4)	0.31	0.7(0.3-2.2)	0.7

Male	0.6(0.2-1.6)	0.4	1.1(0.4-2.6)	ns
Tobacco	1.1(0.4-2.9)	0.1	0.8(0.3-2.1)	0.8
Age≥60	1.6(0.6-4.1)	0.5	1.4(0.6-3.7)	ns
LVEF≤45%	0.6(0.12-3.3)	0.7	3.9(1.2-12.2)	<0.05
Black Race	2.4(0.7-7.8)	0.2	1.8(0.6-5.5)	0.3

REFERENCES

1.Willerson JT, Cohen. Cardiovascular Medicine.

2. Fitzerald DJ, Louis R, Catella F, Fitzerald GA. Platelet activation in unstable coronary disease. *NEJM* 315:983-9, 1986.

3.Willerson JT et al. Conversion from chronic to acute coronary artery disease: Sp[eculation regarding mechanisms. *American Journal of Cardiology* 54: 1349-54, 1981.

4. Buja ML, Willerson JT: Clinicopathologic correlates of acute ischemic heart diseasesyndromes. *American Journal of Cardiology* 47:343-356, 1981.

5. Willerson JT et al. Specific platelet mediators and unstable coronary artery lesions. Experimental evidence and potential clinical implications. *Circulation* 80,1:198-205, 1989.

6. Lee, TH, Rouan GW, Weisberg MC et al. Clinical characteristics and natural history of patients with acute myocardial infarction sent home from the emergency room. *Ann Emerg Med* 16: 1145-50, 1987.

7. McCarthy BD, Behansky JR, D'Agostino RB, Selker HP. Missed diagnosis of acute myocardial infarction in the emergency department : results from a multicenter study. *Ann Emerg Med* 22: 579-82, 1993

8. Kontos MC, Jesse RL, Schmidt KI, Ornato JP, Tatum JI. Value of acute rest sestamibi perfusion imaging for evaluation of patients admitted to the emergency department with chest pain. *JACC* 30: 976-82.

9. Varetto T, Cantalupi, Altieri A, Orlandi C. Emergency room technetium-99m sestamibi imaging to rule out acute myocardial ischemic events in patients with nondiagnostic electrocardiograms. *JACC* 22: 1804-1808, 1993.

10. Hilton TC, Thompson RC, Williams HJ, Saylors R, Fulmer H. Stowers SA. Technetium-99m sestamibi myocardial perfusion imaging in the emergency room evaluation of chest pain. *JACC* 23 1016-22, 1994

11. Ohman EM, Armstrong PW, Christenson RH et al. (GUSTO IIA Investigators) Cardiac troponin T levels for risk stratification acute myocardial ischemia. *NEJM* 335:133-41, 1996.

12. Trip MD, Volkert MC, Van Capelle FJL, Vreeken J. Platelet hyperactivity and prognosis in survivors of myocadial infarcion. *NEJM* 322: 1549-54, 1990.

13. Ikeda H, Nakayama H, Oda T, Kuwano K, Muraishi A, Sugi K, Koga Y, Toshima H. Soluble form of P-selectin in patients with acute mycardial infarcion. *Coronary Artery Disease* June;5: 515-8, 1994.

14. Shyu KG, Chang H, Lin CC, Kuan P. Circulating intercellular adhesion molecule-1 and E-selectin in patients with acute coronary syndrome. *Chest* 109:1627-30, 1996.

15. Carr ME, Zekert SL. Measurement of Platelet Mediated-Force Development during Plasma Clot Formation. *Am J Med Sci* 302:13-18,1991.

16. Carr ME: Measurement of platelet force: the Hemodyne® hemostasis analyzer. *Clin Lab Management Rev* 9:312-319,1995.

17. Carr ME, Zekert SL. Abnormal clot retraction, altered fibrin structure, and normal platelet function in multiple myeloma. *Am J Physiol (Heart Circ. Physiol. 35)* 266:H1195-H1201,1994.

18. Carr ME, Zekert SL. Force monitoring of clot retraction during DDAVP therapy for the qualitative platelet disorder of uraemia: Report of a case. *Blood Coagulation and Fibrinolysis* 2:303-308,1991.

19. Greilich PE, Carr ME, Carr SL, Chang AS. Reductions in Platelet Force Development by Cardiopulmonary Bypass Are Associated with Hemorrhage. *Anesthes Analges* 80:459-465,1995.

20. Alving BM, Reid TJ, Fratantoni JC, Finlayson JS. Frozen platelets and platelet substitutes in transfusion medicine. *Transfusion* 37:866-876,1997.

21. Reid T, Snider R, Hartman K, Greilich PE, Carr ME, Alving BM. A Method for the quantitative assessment of platelet-induced clot retraction and clot strength in fresh and stored platelets. *Vox Sang* 75:270-277,1998.

22. Carr ME, Carr SL, Hantgan RR, Braaten J: Glycoprotein IIb/IIIa Blockade Inhibits Platelet Mediated Force Development and Reduces Gel Elastic Modulus. *Thromb Haemostas* 73:499-505,1995Greilich PE, Carr ME, Carr SL, Chang AS: Reductions in Platelet Force

23. Greilich PE, Carr ME, Zekert SL, Dent RM: Quantitative Assessment of Platelet Function and Clot Structure in Patients with Severe Coronary Artery Disease. *Am J Med Sci* 307:15-20,1994.

24. Tatum JL, Jesse RL, Kontos MC, et al. Comprehensive strategy for the evaluation and triage of the chest pain patient. *Ann Emerg Med* 29:116-23, 1997.

While the invention has been described in terms of its preferred embodiments, those skilled in the art will recognize that the invention can be practiced with modification within the spirit and scope of the appended claim.

CLAIMS

We claim:

1. A method for identifying patients at risk for atherosclerosis, comprising the steps:

obtaining a measurement on the blood sample of a patient selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement to a control to identify a patient as being at risk for atherosclerosis, wherein said patient is identified to be at risk when said measurement is elevated relative to said control.

2. The method of claim 1 wherein said measurement is for platelet contractile force and said control is a value ranging from approximately 5.4 to 8.4 kilodynes.

3. The method of claim 1 wherein said measurement is for clot elastic modulus and said control is a value ranging from approximately 18 to 26 kilodynes per cm^2.

4. The method of claim 1 wherein said step of obtaining is performed by measuring clot contraction forces exerted during clot formation.

5. A method for identifying patients having a bleeding risk, comprising the steps:

obtaining a measurement on the blood sample of a patient selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement to a control to identify a patient as being at risk for a bleeding risk, wherein said patient is identified to be at risk when said measurement is reduced relative to said control.

6. The method of claim 5 wherein said measurement is for platelet contractile force and said control is a value ranging from approximately 5.4 to 8.4.

7. The method of claim 5 wherein said measurement is for clot elastic modulus and said control is a value ranging from approximately 18 to 26 kilodynes per cm^2.

8. The method of claim 5 wherein said step of obtaining is performed by measuring clot contraction forces exerted during clot formation.

9. A method of monitoring treatment or therapy of a patient suffering from unstable angina or myocardial infarction, comprising the steps of:

obtaining a baseline measurement on a blood sample taken from said patient selected from the group consisting of platelet contractile force and clot elastic modulus;

providing said patient with treatment or therapy;

obtaining a measurement on said blood sample after said step of providing, said measurement being selected from the group consisting of platelet contractile force and clot elastic modulus; and

comparing said measurement and said baseline measurement, wherein progress of said treatment or therapy is indicated by a decline in said measurement relative to said baseline measurement.

10. The method of claim 9 wherein said measurement and said baseline measurement both provide platelet contractile force values.

11. The method of claim 9 wherein said measurement and said baseline measurement both provide clot elastic modulus values.

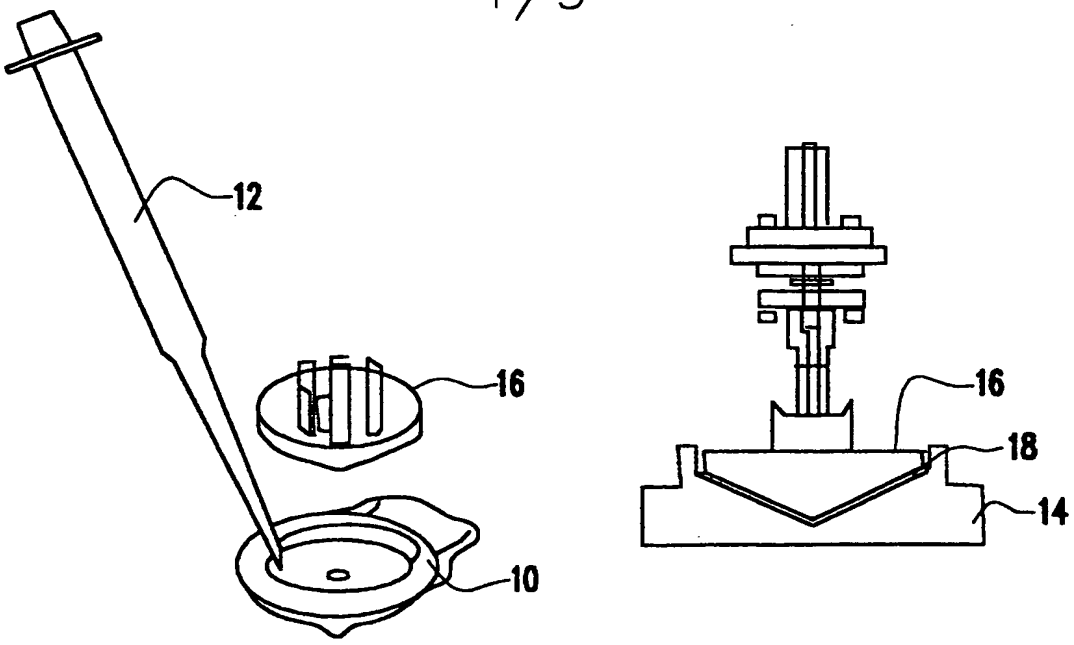
12
16
10
16
18
14

FIG.1

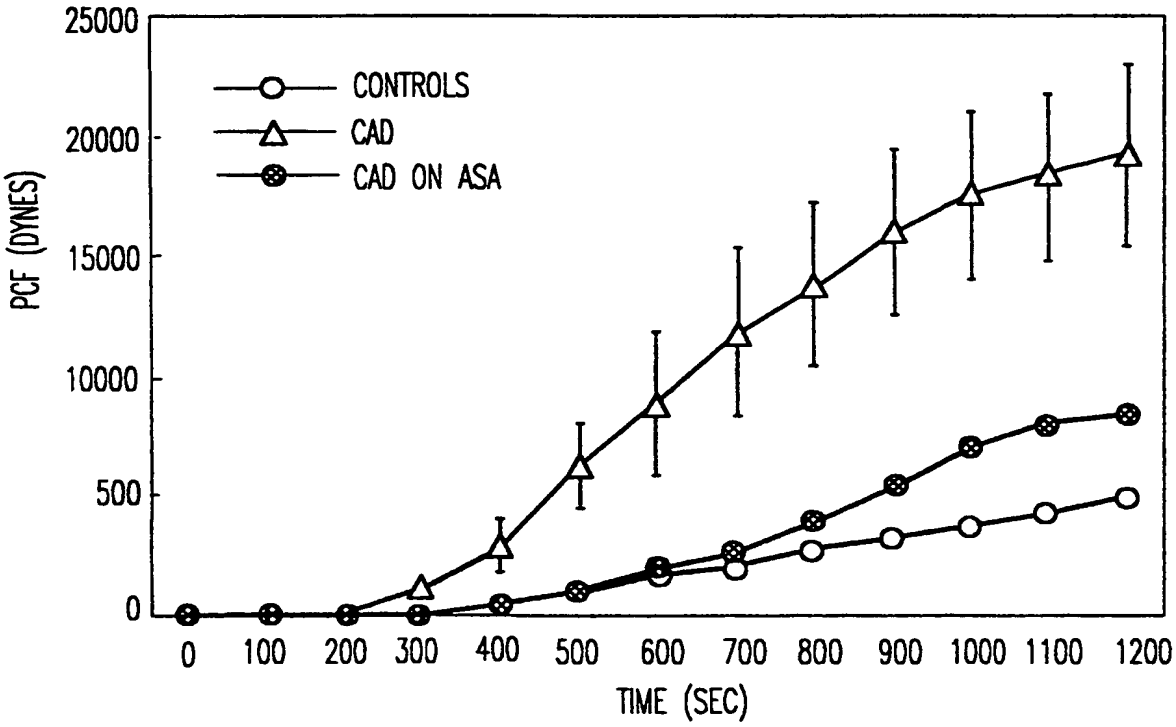
25000
20000
15000
10000
500
0
PCF (DYNES)
CONTROLS
CAD
CAD ON ASA
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
TIME (SEC)

FIG.2

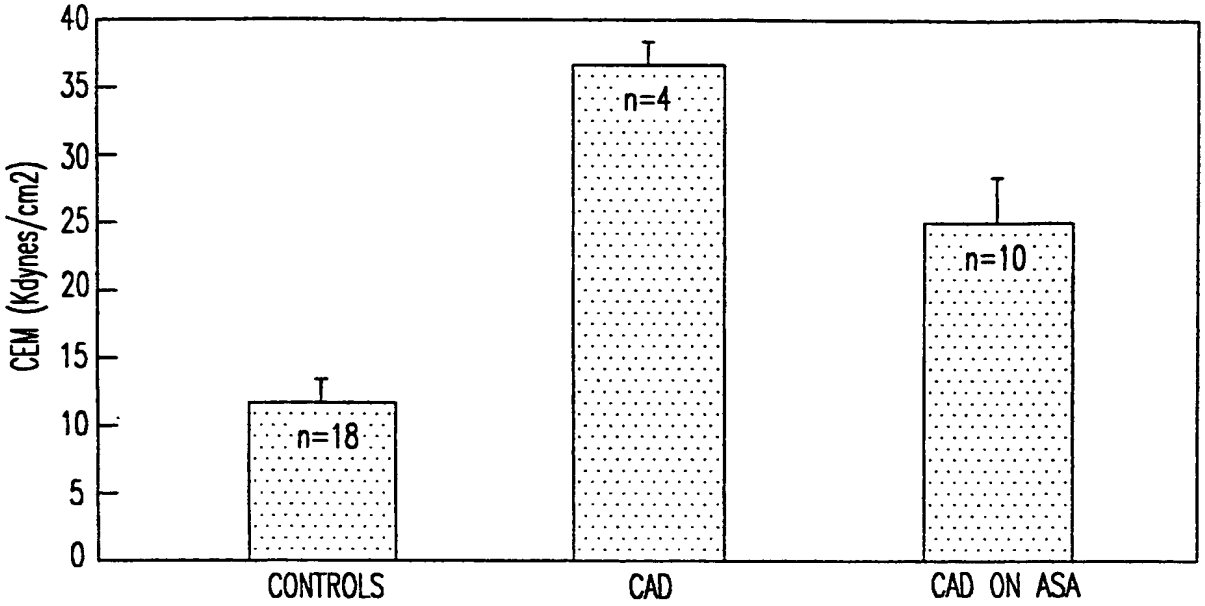
CEM (Kdynes/cm2)
40
35
30
25
20
15
10
5
0
n=18
n=4
n=10
CONTROLS
CAD
CAD ON ASA

FIG.3

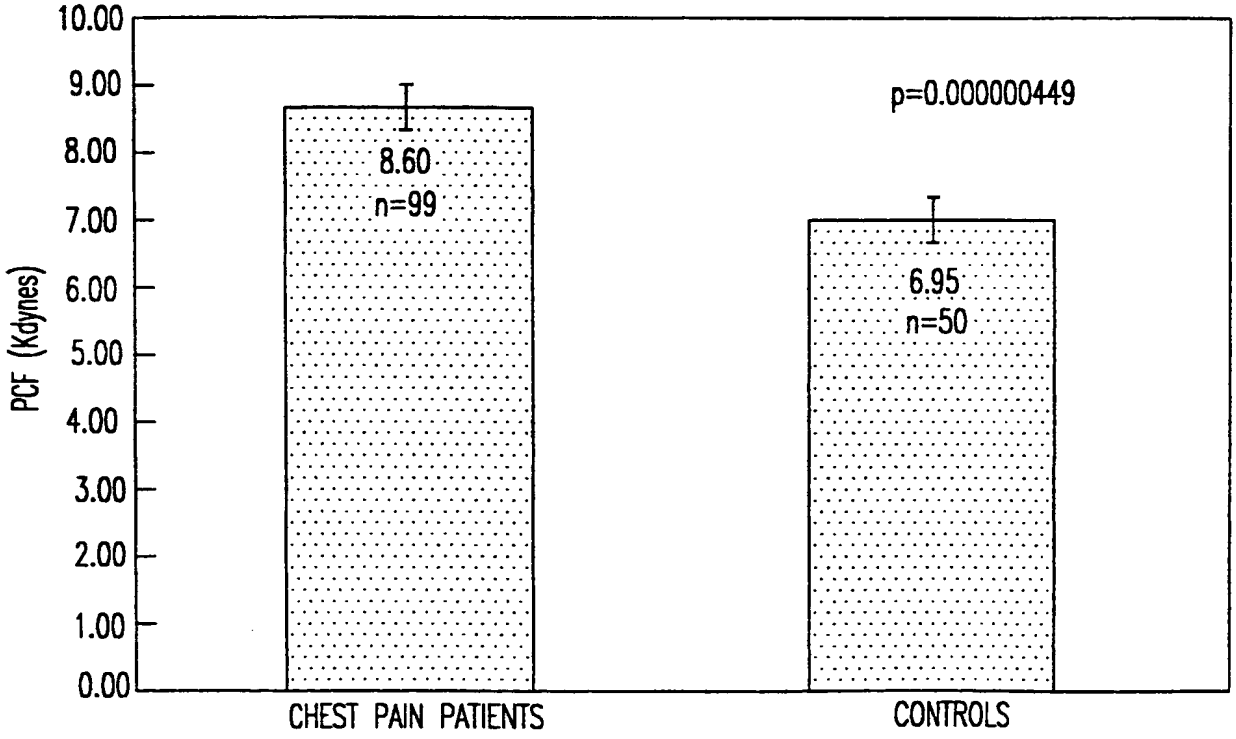
PCF (Kdynes)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
8.60
n=99
p=0.000000449
6.95
n=50
CHEST PAIN PATIENTS
CONTROLS

FIG.4

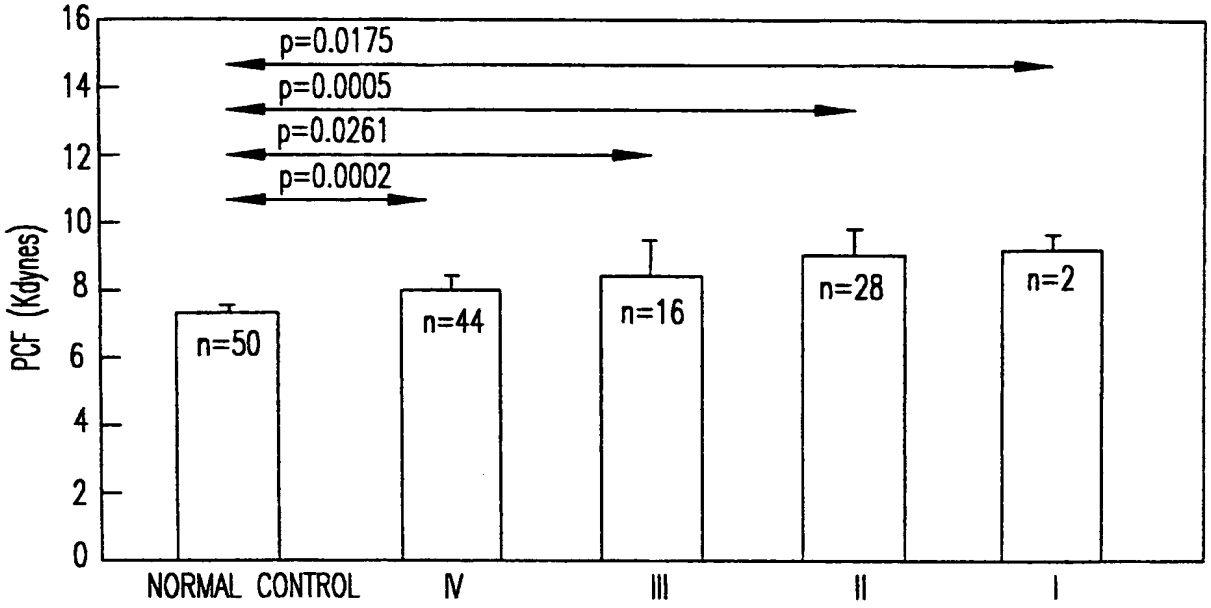
PCF (Kdynes)
16
14
12
10
8
6
4
2
0
p=0.0175
p=0.0005
p=0.0261
p=0.0002
n=50
n=44
n=16
n=28
n=2
NORMAL CONTROL
IV
III
II
I

FIG.5

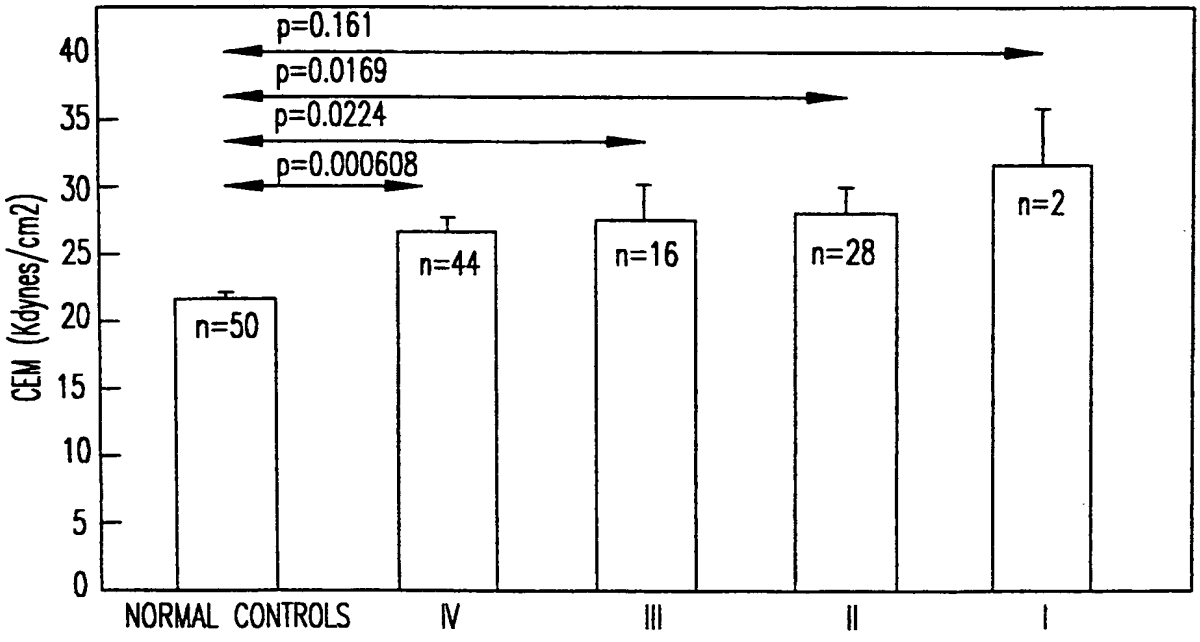
CEM (Kdynes/cm2)
40
35
30
25
20
15
10
5
0
p=0.161
p=0.0169
p=0.0224
p=0.000608
n=50
n=44
n=16
n=28
n=2
NORMAL CONTROLS
IV
III
II
I

FIG.6

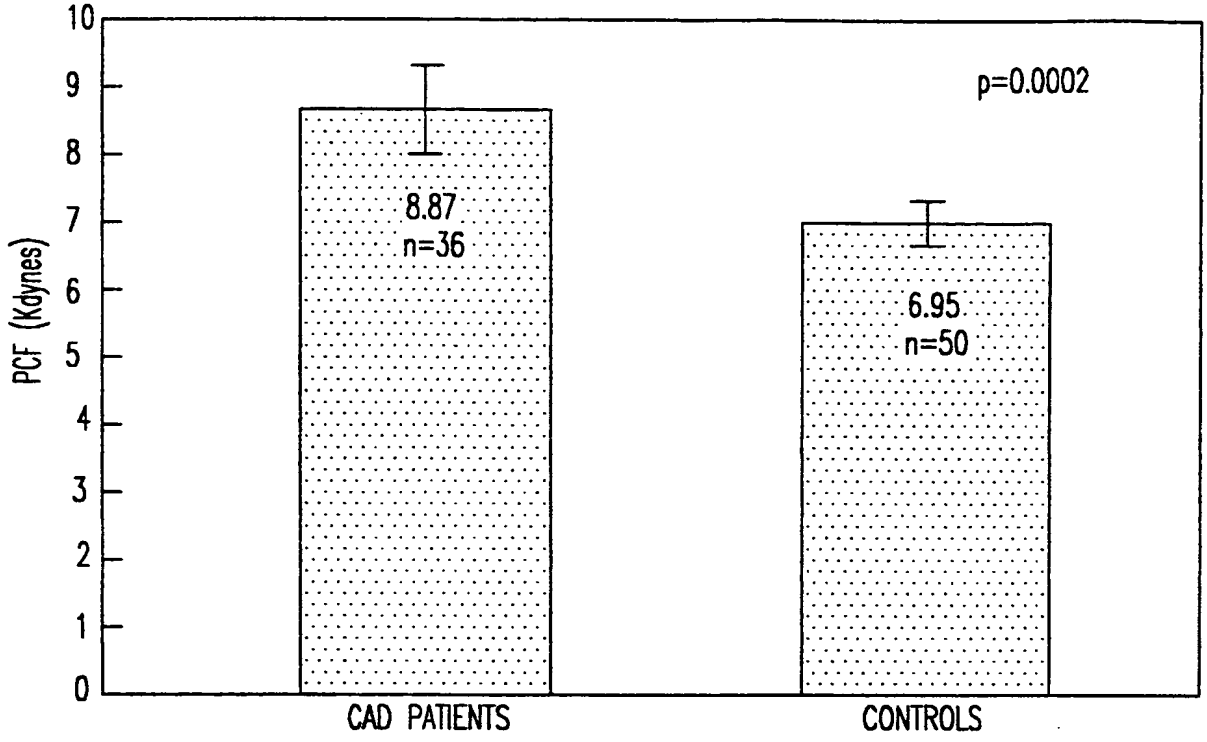
10
9
8
7
6
5
4
3
2
1
0
PCF (Kdynes)
p=0.0002
8.87
n=36
6.95
n=50
CAD PATIENTS
CONTROLS

FIG.7

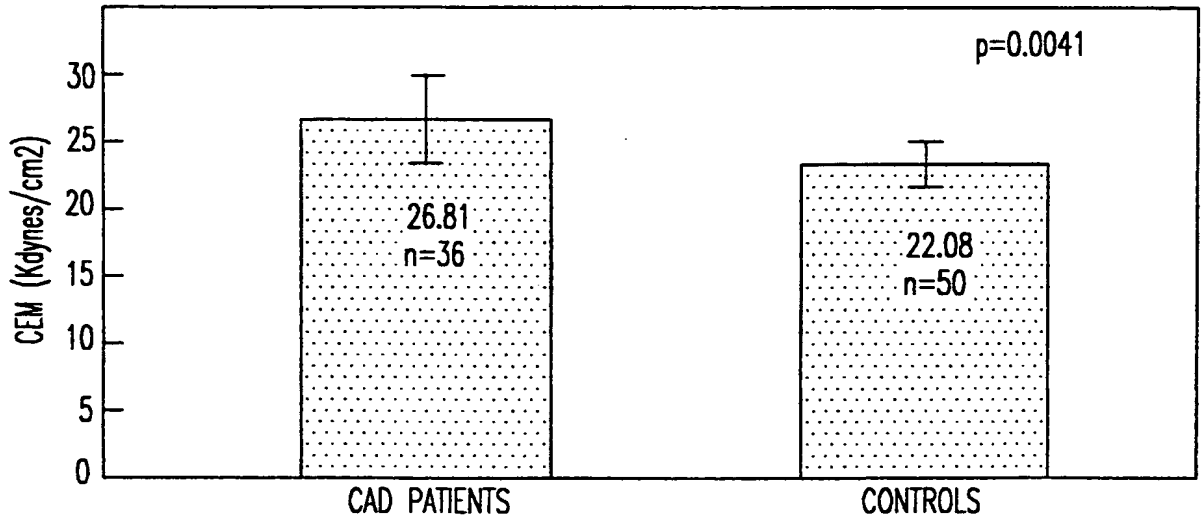
30
25
20
15
10
5
0
CEM (Kdynes/cm2)
p=0.0041
26.81
n=36
22.08
n=50
CAD PATIENTS
CONTROLS

FIG.8

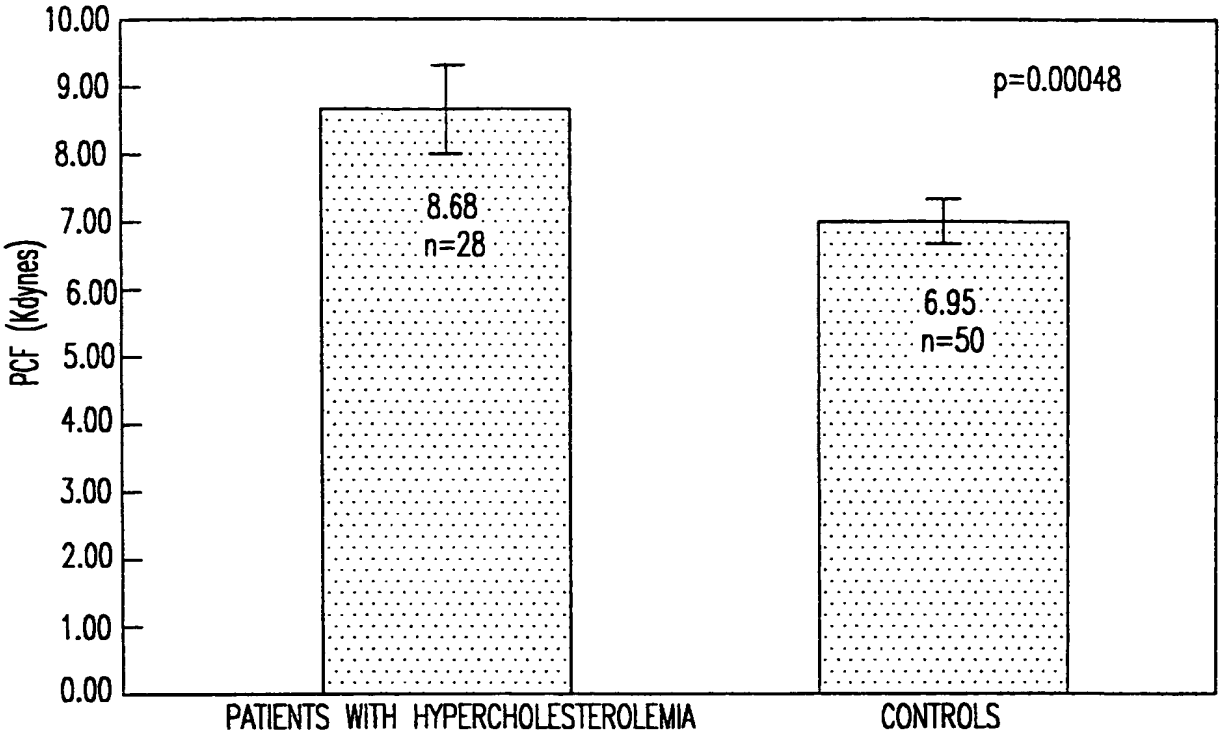
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
PCF (Kdynes)
p=0.00048
8.68
n=28
6.95
n=50
PATIENTS WITH HYPERCHOLESTEROLEMIA
CONTROLS

FIG.9

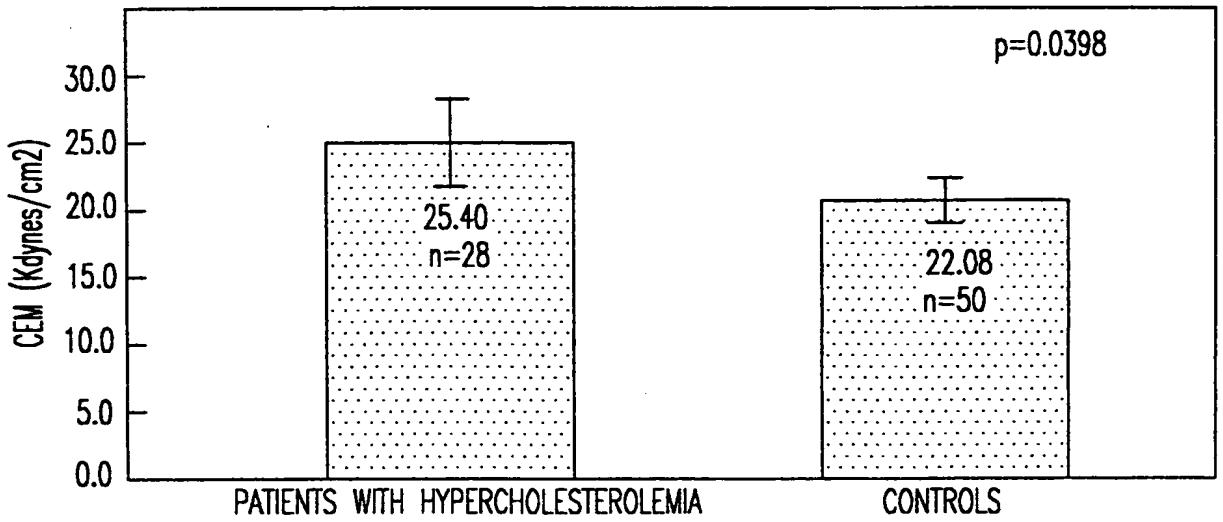
30.0
25.0
20.0
15.0
10.0
5.0
0.0
CEM (Kdynes/cm2)
p=0.0398
25.40
n=28
22.08
n=50
PATIENTS WITH HYPERCHOLESTEROLEMIA
CONTROLS

FIG.10

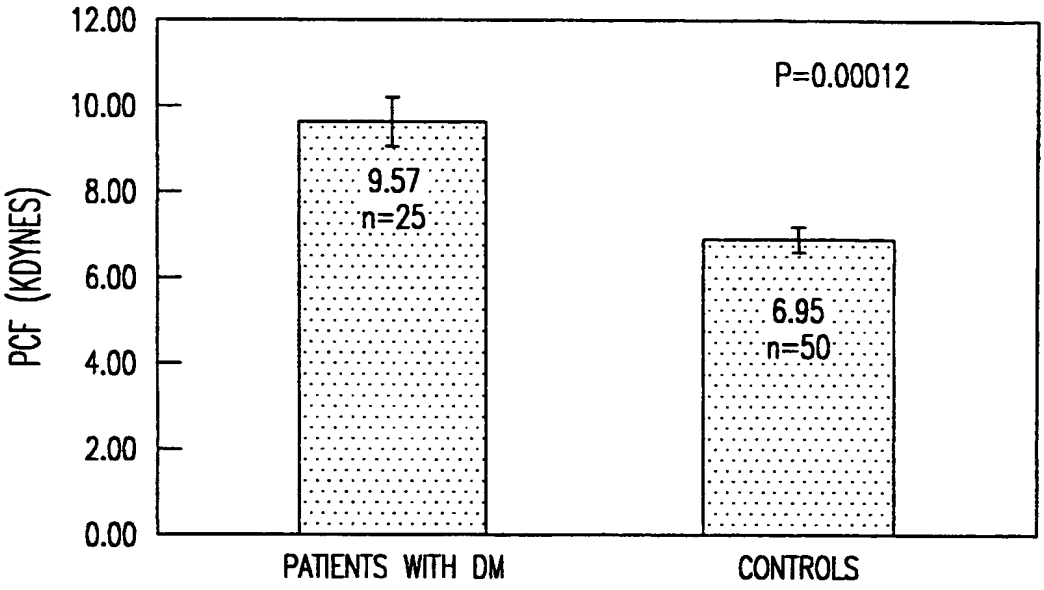
12.00
10.00
8.00
6.00
4.00
2.00
0.00
PCF (KDYNES)
P=0.00012
9.57
n=25
6.95
n=50
PATIENTS WITH DM
CONTROLS

FIG. 11

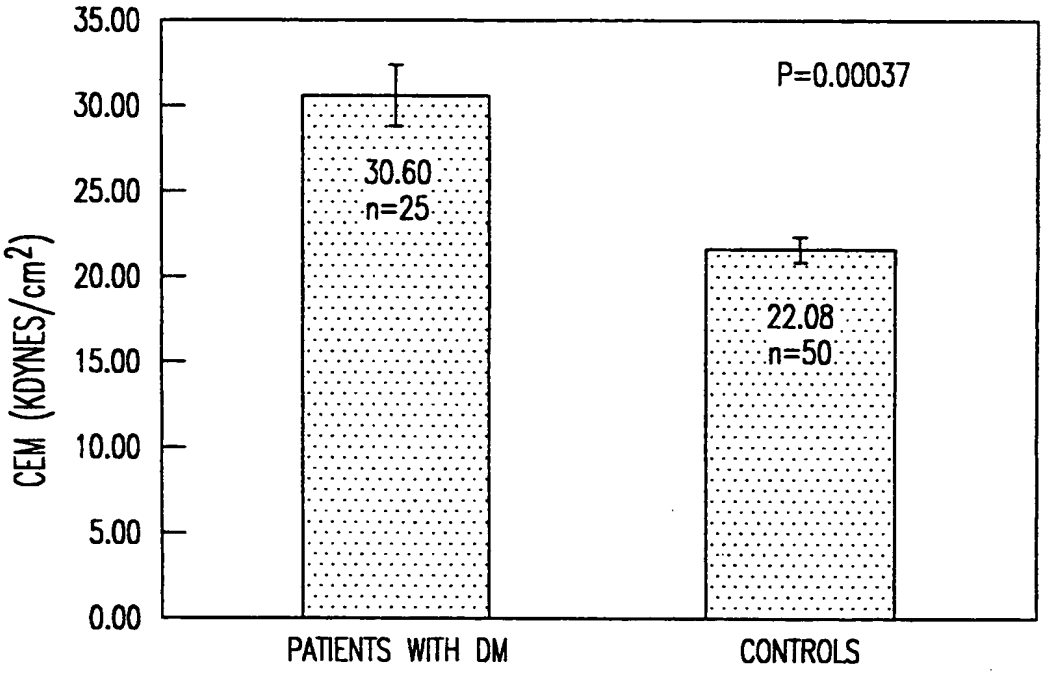
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
CEM (KDYNES/cm2)
P=0.00037
30.60
n=25
22.08
n=50
PATIENTS WITH DM
CONTROLS

FIG. 12

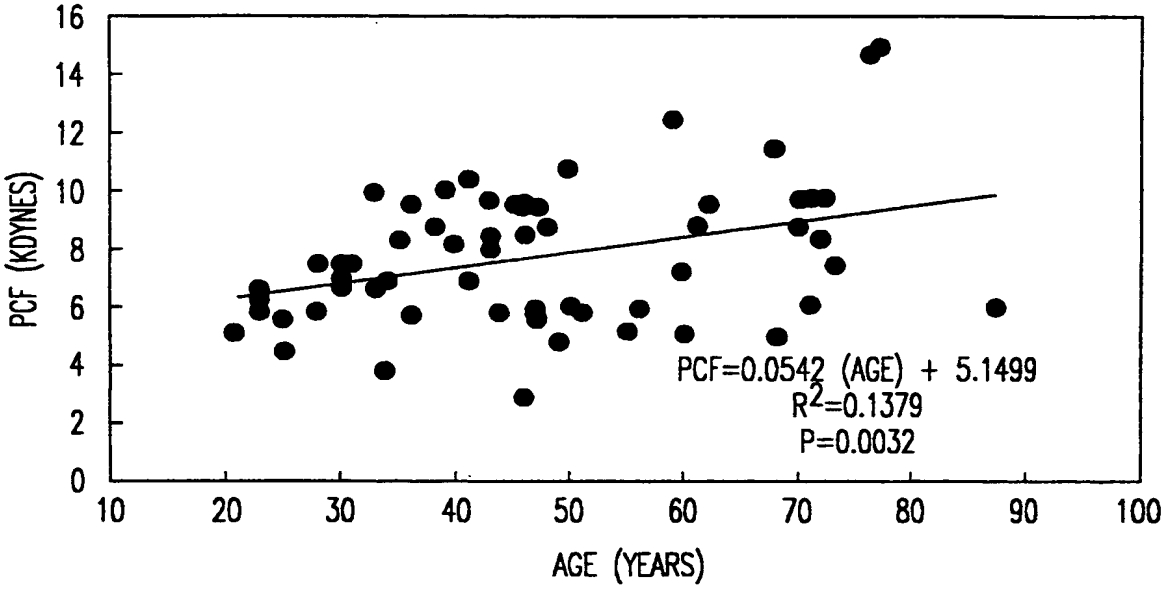
PCF (KDYNES)
AGE (YEARS)
PCF=0.0542 (AGE) + 5.1499
R^2=0.1379
P=0.0032

FIG. 13

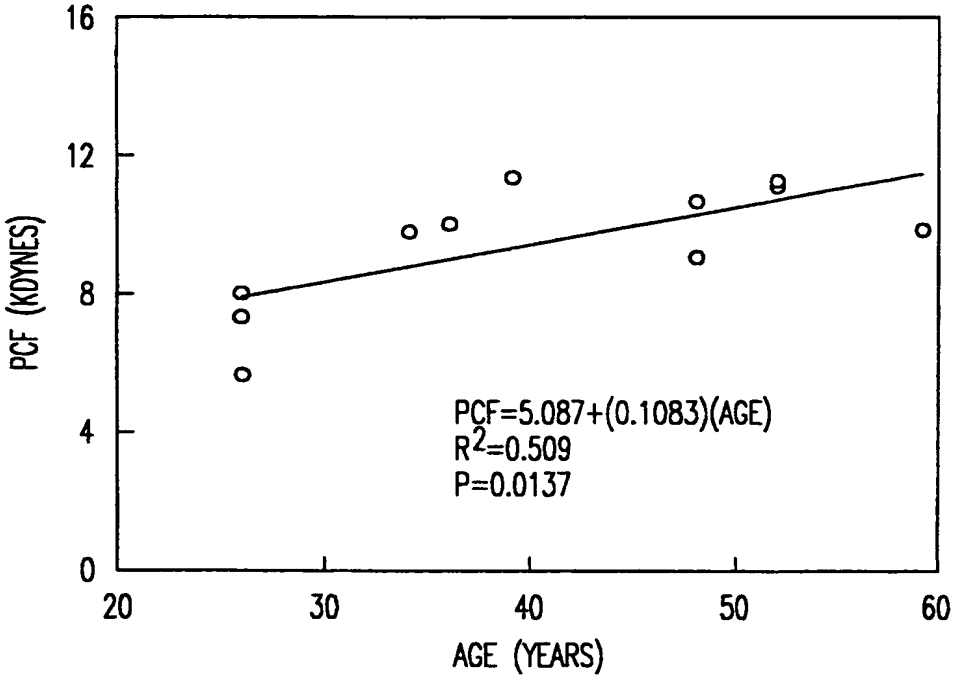
PCF (KDYNES)
AGE (YEARS)
PCF=5.087+(0.1083)(AGE)
R^2=0.509
P=0.0137

FIG. 14

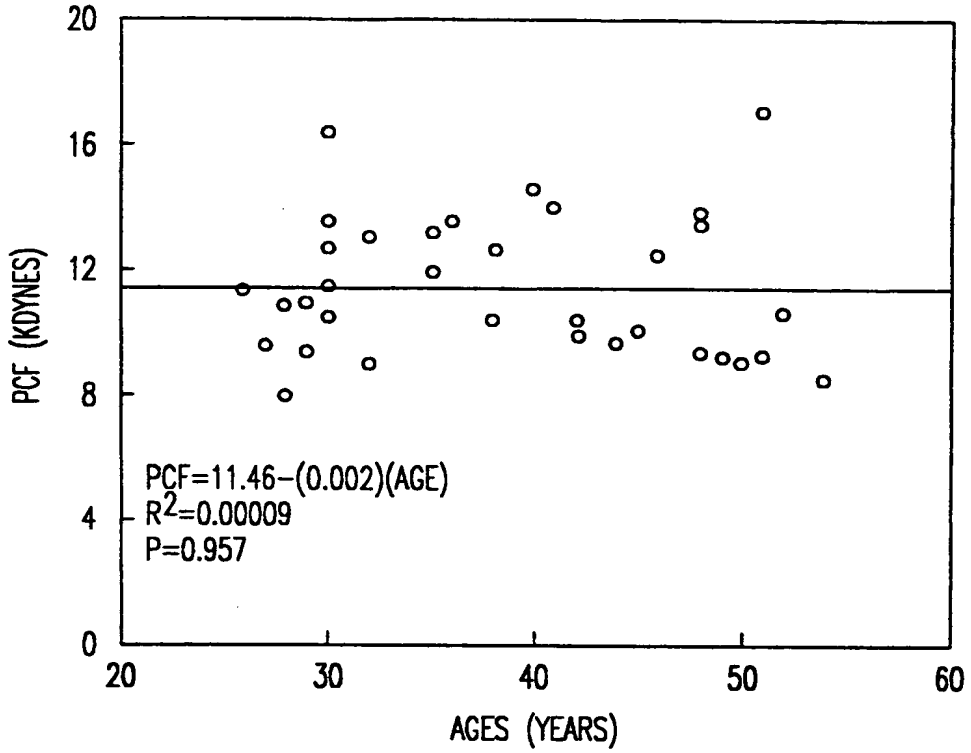
20
16
12
8
4
0
PCF (KDYNES)
PCF=11.46-(0.002)(AGE)
R2=0.00009
P=0.957
20
30
40
50
60
AGES (YEARS)

FIG. 15

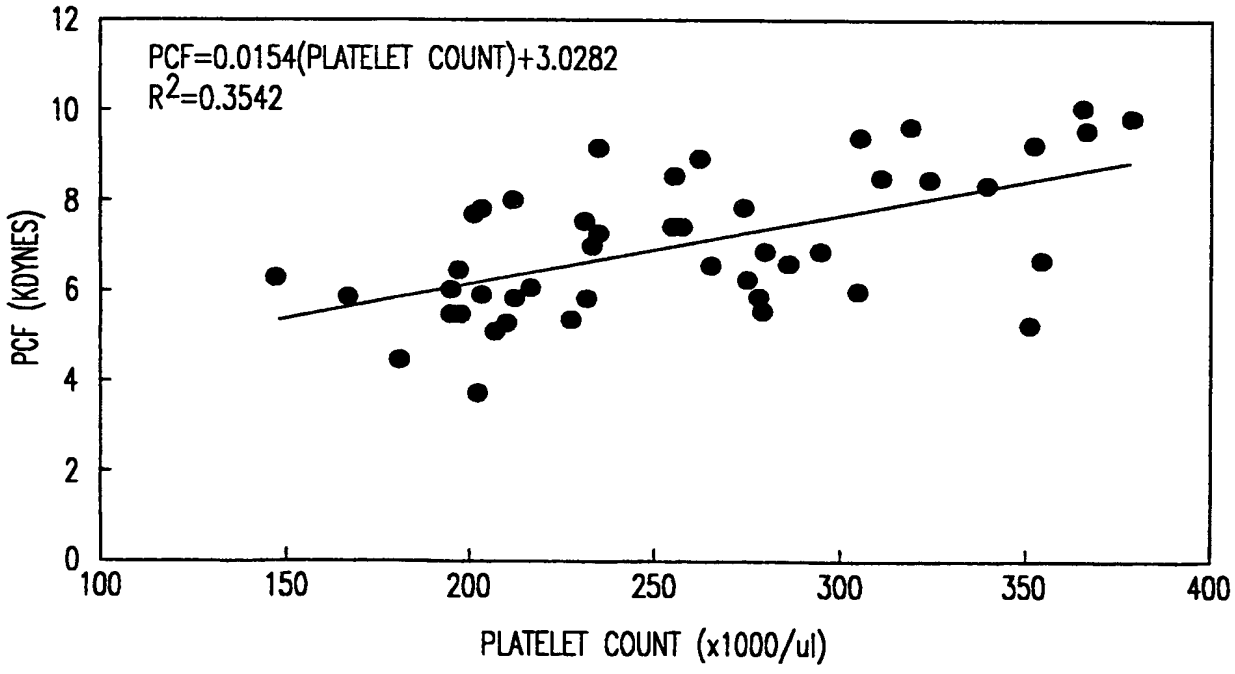
12
10
8
6
4
2
0
PCF (KDYNES)
PCF=0.0154(PLATELET COUNT)+3.0282
R2=0.3542
100
150
200
250
300
350
400
PLATELET COUNT (x1000/ul)

FIG. 16

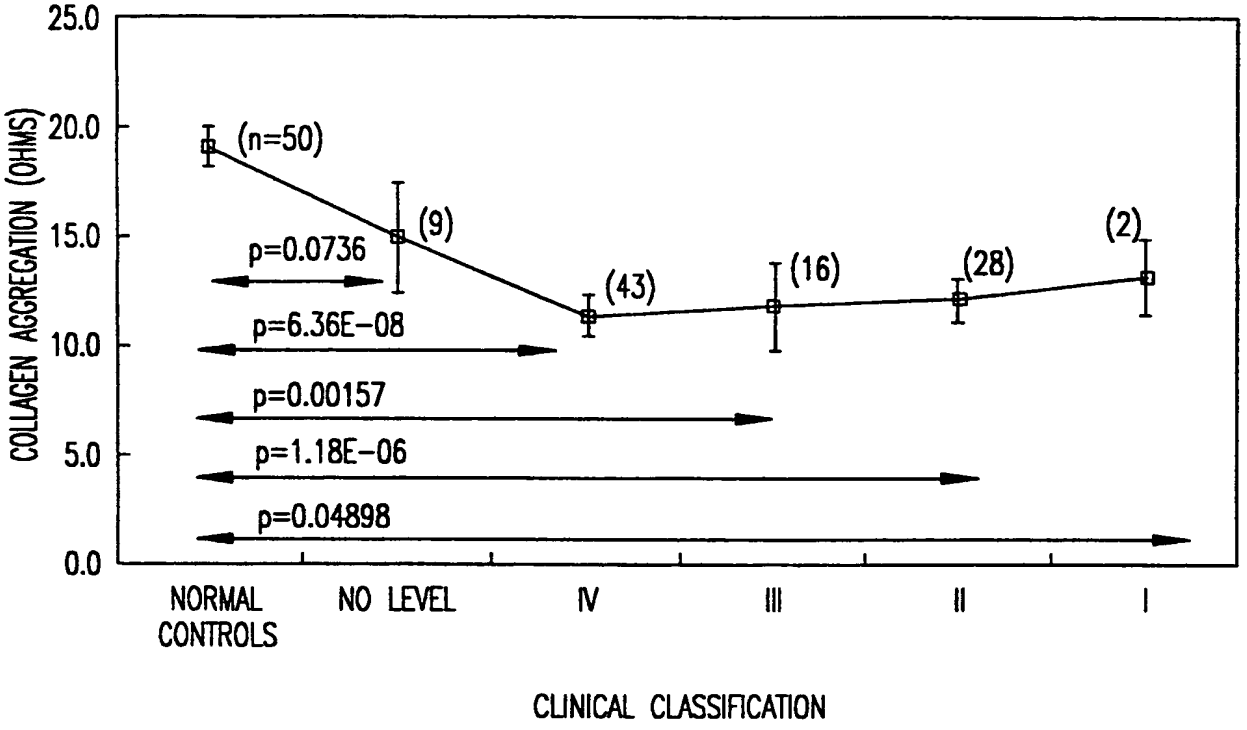
25.0
20.0
15.0
10.0
5.0
0.0
COLLAGEN AGGREGATION (OHMS)
(n=50)
(9)
(43)
(16)
(28)
(2)
p=0.0736
p=6.36E-08
p=0.00157
p=1.18E-06
p=0.04898
NORMAL CONTROLS
NO LEVEL
IV
III
II
I
CLINICAL CLASSIFICATION

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21848

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 38/00, 38/48; C12Q 1/56, 1/68; G01N 33/86
US CL :424/94.64; 435/6, 13; 436/69; 514/2, 12, 18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.64; 435/6, 13; 436/69; 514/2, 12, 18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,205,159 A (CARR, JR.) 27 April 1993.	1-11
A	US 5,293,772 A (CARR, JR.) 15 March 1994.	1-11
A	US 5,691,160 A (JANMEY et al.) 25 November 1997.	1-11
A	CARR, M. E. Fibrin Structure and Concentration Alter Clot Elastic Modulus But Do Not Alter Platelet Mediated Force Development. Blood Coagulation and Fibrinolysis. 1995, Vol. 6, No. 1, pages 79-86.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
14 NOVEMBER 2000	27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/21848

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	REID, T. J. A Method for the Quantitative Assessment of Platelet Induced Clot Retraction and Clot Strength in Fresh and Stored Platelets. Vox Sanguinis. 1998, Vol. 75, No. 4, pages 270-277.	1-11